

Claims

1. A nucleotide sequence of expression cassette OXY-1 of SEQ ID No. 1.
2. A modified staphylokinase SAK-2 gene of SEQ ID No. 2.
3. A peptide sequence of modified staphylokinase SAK-2 gene, of SEQ ID No. 3.
- 5 4. A plasmid pRM1 having International Deposition No. BPL-0019.
5. A plasmid pOXYSAK-1 having International Deposition No. BPL-0020.
6. A plasmid pOXYSAK-2 having International Deposition No. BPL-0021.
7. A recombinant *E. Coli* of International Deposition No. 5146, the International Depository is "Microbial Type Culture Collection" at Institute of Microbial Technology, Chandigarh, 10 India, having a plasmid pRM1 of International Deposition No. BPL-0019.
8. A recombinant *E. Coli* of International Deposition No. 5147, the International Depository is "Microbial Type Culture Collection" at Institute of Microbial Technology, Chandigarh, India, having a plasmid pOXYSAK-1 of International Deposition No. BPL-0020.
9. A recombinant *E. Coli* of International Deposition No. 5148, the International Depository is "Microbial Type Culture Collection" at Institute of Microbial Technology, Chandigarh, 15 India, having a plasmid pOXYSAK-2 of International Deposition No. BPL-0021.
10. A process for over-producing staphylokinase and its analogues by modulating level of oxygen of its growth medium in a host system, said method comprising steps of:
 - 20 a. preparing a piece of DNA carrying genetic information for the production of staphylokinase,
 - b. modifying 10 amino-terminal residues of SAK encoding DNA, wherein Lys6 and Lys8 residues of SAK are changed to small neutral amino-acid residues,
 - c. constructing DNA expression cassette OXY-1,
 - d. integrating piece of DNA obtained at step (a) or step (b) with the OXY-1 to 25 obtain pOXYPRO,
 - e. transferring integrated product of step (d) on a plasmid vector to obtain plasmid construct pOXYSAK-1, and pOXYSAK-2 respectively,
 - f. introducing the plasmid constructs of step (e) into a host systems,
 - g. culturing the host cell for over-production of SAK or its derivatives under high aeration and changing level of oxygen below 5% of atmospheric oxygen 30 level when cell growth reaches to exponential phase to obtain cell mass,
 - h. lysing the cells of step (g) to separating cell lysate from the cellular debris, and thereby obtaining the staphylokinase and its analogues.

11. A process as claimed in claim 10, wherein the Lys6 and Lys8 residues of SAK are changed into small and neutral amino acid residues.
12. A process as claimed in claim 10, wherein the plasmid vector is a high or medium copy number plasmid.
- 5 13. A process as claimed in claim 10, wherein the host system is selected from a group comprising *E. coli*, *Bacillus*, and Yeast.
14. A process as claimed in claim 10, wherein the sequence of OXY-1 is modified depending upon the host system.
15. A process as claimed in claim 10, wherein the amino acids are selected from a group comprising Alanine, and Glycine.
- 10 16. A process as claimed in claim 10, wherein the growth medium is Luria Broth (LB) medium.
17. A process as claimed in claim 10, wherein culturing the host cell for over-production of SAK or its derivatives at shake flask culture or at fermentation.
- 15 18. A process as claimed in claim 17, wherein culturing the host cell till O.D. 600 reaches 0.6 to 0.7.
19. A process as claimed in claim 17, wherein fermentation is a two-stage fed-batch fermentation.
20. A process as claimed in claim 10, wherein obtaining the cell mass by centrifugation or filtration.
- 20 21. A process as claimed in claim 10, wherein lysing the cells by method selected from a group comprising sonication, chemical, and mechanics lysis.
22. A process as claimed in claim 10, wherein separating the cell lysate from the cellular debris by centrifugation.
23. A method of dissolving blood clot in a subject in need thereof, said method comprising step of administering pharmaceutically effective amount of streptokinase analogue SAK-2, optionally along with additive(s).
- 25 24. A method as claimed in claim 23, wherein the additive is selected from a group comprising nutrients consisting of proteins, carbohydrates, sugar, talc, magnesium stearate, cellulose, calcium carbonate, starch-gelatin paste, and/or pharmaceutically acceptable carrier, excipient, diluent, or solvent.
- 30 25. A method as claimed in claim 23, wherein the SAK-2 and additives are in a ratio ranging between 1:10 to 10:1.